

AMENDMENT TO THE CLAIMS

1. (Currently Amended) A method for imaging tissue, comprising the steps of:

mounting the tissue on a computer controlled stage of a microscope;

determining volumetric imaging parameters;

directing at least two ~~photons~~ photon excitation light onto a region of interest;

scanning the ~~region of interest~~ excitation light across a first portion of the tissue;

imaging a plurality of layers of the tissue in a ~~plurality of volumes~~ first volume of the tissue in the region of interest to provide first image data;

sectioning the first portion of the tissue ~~and~~;

scanning the excitation light across a second portion of the tissue;

imaging a second plurality of layers of the tissue in a second ~~plurality of volume~~ of the tissue ~~in the region of interest~~ to provide second image data;

~~detecting an image of the tissue due to said excitation light~~, and

processing three-dimensional the first image data and the second image data that is imaged to create form a three-dimensional image of the region of interest tissue.

2. (Original) The method of Claim 1, wherein the microscope comprises a multi-photon microscope.

3. (Currently Amended) The method of Claim 1, ~~wherein the detected image is~~ further comprising detecting a fluorescent image.

4. (Original) The method of Claim 1, wherein the image is a confocal reflectance image.
5. (Currently Amended) The method of Claim 2, wherein the excitation light has a penetration depth in the tissue of the multi-photon microscope is in the range of approximately 200-500  $\mu\text{m}$ .
6. (Currently Amended) The method of Claim 1, wherein the step of sectioning further comprises operating a microtome system that is integral with the microscope.
7. (Currently Amended) The method of Claim 1, wherein ~~the speed of~~ the step of imaging a plurality of layers further comprises detecting at least 5 image frames per second.
8. (Original) The method of Claim 1, wherein the step of scanning further comprises video rate scanning.
9. (Original) The method of Claim 1, further comprising providing a depth resolution of approximately 0.1 to 2  $\mu\text{m}$ .
10. (Original) The method of Claim 1, wherein the step of scanning further comprises a low resolution mode and a high resolution mode.
- 11-23 (Cancelled)
24. (Currently Amended) A method of imaging tissue in-vivo, comprising the steps of:  
mounting the tissue in a multi-photon microscope;

directing at least two ~~photons~~ photon excitation light onto a region of interest;

scanning a plurality of layers of the tissue in the region of interest;

imaging a plurality of layers in the tissue in the region of interest;

detecting a fluorescence image of the region of interest ~~due~~ in response to said excitation light; and

processing the detected fluorescence image comprising the steps of:

sequentially storing a plurality of ~~portions of three-dimensional image data-set~~ sets;

enhancing the image data ~~set~~ sets;

registering ~~individual~~ the plurality of three-dimensional data sets to generate a large three-dimensional data set; and  
displaying the three-dimensional data set of the region of interest.

25. (Original) The method of Claim 24, wherein the step of processing further comprises compressing the three-dimensional data set.

26. (Original) The method of Claim 24, wherein the step of processing further comprises identifying and quantifying features of the region of interest.

27. (Original) The method of Claim 24, wherein the step of processing further comprises analyzing the three-dimensional data set.

28. (Original) The method of Claim 24, wherein the step of imaging further comprises imaging mitotic recombination in the tissue.

29. (Original) The method of Claim 24, wherein the step of scanning further comprises a low resolution mode and a high resolution mode.

30. (New) The method of Claim 1 wherein the sectioning step comprises moving the stage from an imaging position to a sectioning position, removing a layer of tissue with a sectioning tool, and moving the stage to the imaging position.

31. (New) The method of Claim 30 wherein the removing step further comprises cutting the layer of tissue with a blade.

32. (New) The method of Claim 30 wherein the moving step comprises translating the stage in an X-Y plane and elevating the stage to position the tissue relative to the sectioning tool.

33. (New) The method of Claim 1 further comprising performing a plurality of sectioning steps to remove successive layers of tissue.

34. (New) The method of Claim 1 further comprising programming a computer to control an imaging sequence and a stage translation sequence.

35. (New) The method of Claim 1 further comprising scanning the tissue using a moving mirror.

36. (New) The method of Claim 35 further comprising rotating a mirror relative to a light beam emitted by a laser.

37. (New) The method of Claim 35 further comprising scanning the tissue using a second mirror.

38. (New) The method of Claim 1 further comprising detecting images with an image sensor.

39. (New) The method of Claim 1 further comprising detecting images with a charge coupled device or CMOS imaging device.

40. (New) The method of Claim 1 further comprising detecting light with a photomultiplier tube detector.